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IMPACT OF INFERTILITY AND ASSISTED REPRODUCTIVE TECHNOLOGY ON CANCER RISK

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Impact of infertility and assisted reproductive technology on cancer risk

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ABSTRACT

Ovarian stimulation drugs, in particular those used in assisted reproductive technology (ART) treatments for infertility, have been suspected to influence cancer risk. In addition, infertility per se may be a risk factor for some cancer types. The focus of this thesis project was to examine the risk of breast and gynecological cancers among infertile women and women treated with ovarian stimulation.

The four studies included in this thesis aimed to investigate: if ovarian stimulation may alter mammographic density (study I), if the use of ART influences the risk of breast cancer (study II), borderline ovarian tumors (BOT) or ovarian cancer (study III), and if infertility is associated with the risk of breast, ovarian or endometrial cancer (study IV). The first study was based on data from a screening-based cohort and the other three studies used information from Swedish population-based registers.

Study I evaluated whether having a history of infertility or ovarian stimulation were associated with mammographic density, which is a strong risk factor for breast cancer. In studies II and III, cancer risk was examined among parous women who gave birth in Sweden between 1982 and 2012, comparing risks in women who had given birth following ART to that of parous women with and without infertility. In study II, breast cancer risk was also evaluated among women born 1960–1992, comparing the risk in women exposed to ovarian stimulation in 2005 or later to that of unexposed women with and without infertility. In study IV, the risk of breast, ovarian and endometrial cancer was assessed in women born 1942–1992, comparing the risk in women diagnosed with infertility, ovulatory disturbances or endometriosis to that of women with none of these diagnoses.

Although mammographic density was higher in women with a history of infertility, in particular among women exposed to ART, there was no association between infertility or ovarian stimulation and breast cancer risk. Ovarian cancer risk was higher among women diagnosed with infertility, endometriosis, and in a subpopulation of women with ovulatory disorders. Women who had given birth following ART treatments had a higher risk of ovarian cancer and BOT, which was partly explained by infertility. Endometrial cancer risk was higher among women diagnosed with infertility or ovulatory disturbances, but not in women with endometriosis.

In conclusion, the findings suggest that infertile women have a higher risk of ovarian and endometrial cancer, but not breast cancer. The possible increase in ovarian tumor risk following ART treatments needs to be investigated further, since the results were based on a small number of exposed cases and may be confounded by the underlying causes of infertility.

SVENSK SAMMANFATTNING

Hormonella fertilitetsbehandlingar används både för att få igång normal ovulation (ägglossning) hos kvinnor med ovulationsrubbningar och för att få flera äggceller att mogna samtidigt inför assisterad befruktning. Hormonerna som används i dessa behandlingar påverkar i sin tur kroppens egna hormonnivåer och har misstänkts kunna öka risken för att utveckla cancer. Vissa orsaker till infertilitet kan också öka risken för cancer. Den övergripande målsättningen med denna avhandling var att undersöka risken för bröstcancer och gynekologisk cancer hos infertila kvinnor och kvinnor som genomgått hormonella fertilitetsbehandlingar.

Studierna i avhandlingen har undersökt: om hormonella fertilitetsbehandlingar påverkar mammografisk brösttäthet (studie I), om assisterad befruktning påverkar risken för bröstcancer (studie II), cancer eller borderlinetumörer i äggstockarna (studie III) och om infertilitet i sig är kopplat till risken för cancer i bröst, äggstockar eller endometrium (livmoderslemhinna) (studie IV).

Studie I innefattade över 43 000 kvinnor som genomgått mammografiundersökningar samt besvarat en omfattande livsstilsenkät i bröstcancerprojektet Karma. Övriga studier använde information från svenska befolknings- och hälsodataregister. I studie I undersöktes om infertilitet eller fertilitetsbehandlingar påverkade mammografisk brösttäthet, vilket är en stark riskfaktor för cancer. Sambanden mellan assisterad befruktning och cancerrisk undersöktes hos 1,34 miljoner kvinnor som fått sitt första barn mellan 1982 och 2012 i studie II och III. I studie II undersöktes också kopplingen mellan assisterad befruktning, andra hormonella fertilitetsbehandlingar och bröstcancerrisk bland 1,88 miljoner kvinnor födda 1960–1992. Slutligen undersöktes sambanden mellan infertilitet, endometrios, ovulationsrubbningar och cancerrisk hos 2,88 miljoner kvinnor födda 1942–1992 i studie IV.

Resultaten från studie I visade att brösttätheten var högre hos infertila kvinnor, särskilt bland kvinnor som genomgått assisterad befruktning. Däremot var varken infertilitet eller assisterad befruktning kopplat till en högre risk för bröstcancer enligt studie II. Studie IV fann att risken för äggstockscancer var högre hos kvinnor med diagnoserna infertilitet, endometrios eller ovulationsrubbningar. Kvinnor som fött barn efter assisterad befruktning hade också högre risk för äggstockscancer och borderlinetumörer enligt resultaten från studie III, delvis beroende på den underliggande infertiliteten. Studie IV visade också på en ökad risk för endometriecancer hos kvinnor med infertilitet eller ovulationsrubbningar, men inte bland kvinnor med endometrios.

Sammanfattningsvis verkar vissa grupper av infertila kvinnor ha en högre risk för cancer i äggstockar och endometrium men inte bröstcancer. Risken för bröstcancer tycks inte heller påverkas av fertilitetsbehandlingar. Den möjliga ökningen av äggstockstumörer efter assisterad befruktning behöver undersökas närmare, eftersom resultaten baserades på ett litet antal tumörer och risken kan ha påverkats av skillnader i den underliggande infertiliteten mellan kvinnor som fått barn med och utan hjälp av assisterad befruktning.

LIST OF SCIENTIFIC PAPERS

I. Lundberg FE, Johansson ALV, Rodriguez-Wallberg K, Brand JS, Czene K, Hall P and Iliadou AN. **Association of infertility and fertility treatment with mammographic density in a large screening-based cohort of women: a cross-sectional study.** *Breast Cancer Research* 2016;18:36.

II. Lundberg FE, Iliadou AN, Rodriguez-Wallberg K, Bergh C, Gemzell-Danielsson K, Johansson ALV. **Ovarian stimulation and risk of breast cancer in Swedish women.** *Fertility and Sterility* 2017;108(1):137-144.

III. Lundberg FE, Johansson ALV, Rodriguez-Wallberg K, Gemzell-Danielsson K, Iliadou AN. **Assisted reproductive technology and risk of ovarian cancer and borderline tumors in parous women.** *In manuscript.*

IV. Lundberg FE, Iliadou AN, Rodriguez-Wallberg K, Gemzell-Danielsson K, Johansson ALV. **Infertility and risk of breast or gynecological cancer.** *In manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

ART	assisted reproductive technology
ATC	Anatomical Therapeutical Chemical (classification system for pharmaceutical drugs)
BMI	body mass index
CI	confidence interval
COS	controlled ovarian stimulation
FSH	follicle-stimulating hormone
GLM	generalized linear models
GnRH	gonadotropin releasing hormone
hCG	human chorionic gonadotropin
HR	hazard ratio
ICD	International Classification of Diseases
ICD-O	International Classification of Diseases for Oncology
ICSI	intracytoplasmic sperm injection
IVF	*in vitro* fertilization
LH	luteinizing hormone
MBR	Medical Birth Register
MGR	Multi-Generation Register
NBHW	National Board of Health and Welfare (Socialstyrelsen)
NPR	National Patient Register
PCOS	polycystic ovary syndrome
PDR	Prescribed Drug Register
Q-IVF	National Quality Registry for Assisted Reproductive Technology
SCR	Swedish Cancer Register
SIR	standardized incidence ratio
WHO	World Health Organization

1 INTRODUCTION

It has been estimated that one in seven heterosexual couples experience infertility, defined as trying to conceive for at least one year without success (1,2). While some eventually conceive spontaneously, half of these couples go on to seek medical care and about one fifth will receive treatment (3,4).

Hormonal drugs that induce ovulation were developed in the middle of the 20th century and are still commonly used to treat infertility due to anovulation (5,6). The first successful treatment using assisted reproductive technology (ART) was performed in 1978 in the United Kingdom, followed by others in Australia, the United States, Sweden and France between 1980 and 1982 (7). ART treatments introduced new possibilities to treat infertility caused by blocked fallopian tubes in women, low sperm count in men, and unexplained causes. Within a decade, ART became routine procedures in the fertility clinics and the number of treatments has increased dramatically since the middle of the 1990s. Today, around 19,000 ART cycles are performed each year in Sweden, contributing nearly 4% of all births (2).

Ovulation induction and ART treatments include strong hormonal stimulation of the ovaries leading to increased estrogen levels which has in turn been suspected to increase the risk of cancer. In addition, certain causes of infertility are also risk factors for cancers (8). Although a number of studies have investigated cancer risk among women exposed to ovarian stimulation, few have been able to separate potential effects of treatment from that of infertility and the results are inconclusive (9,10).

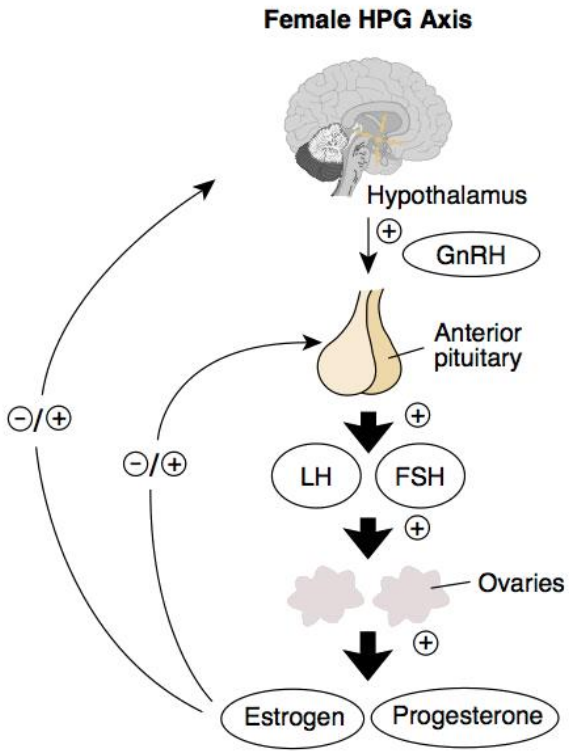
This thesis project aimed to investigate whether infertility or fertility treatments influence the risk of breast and gynecological cancers, using information from Swedish population-based registers as well as a screening-based prospective cohort.

2 BACKGROUND

2.1 REPRODUCTIVE PHYSIOLOGY

The human menstrual cycle is regulated through an intricate system of stimulation and inhibition by hormones secreted in the hypothalamic-pituitary-gonadal axis (Figure 2.1). The hypothalamus releases pulses of gonadotropin-releasing hormone (GnRH), which regulate the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), both from the anterior pituitary. In turn, these two gonadotropins regulate a cyclic function of the ovaries by recruitment of follicles to grow and triggering of ovulation. The hormones secreted by the ovarian follicles during and after these processes (estrogen and progesterone, respectively) induce cyclic growth and maturation of the endometrium inside the uterus (11).

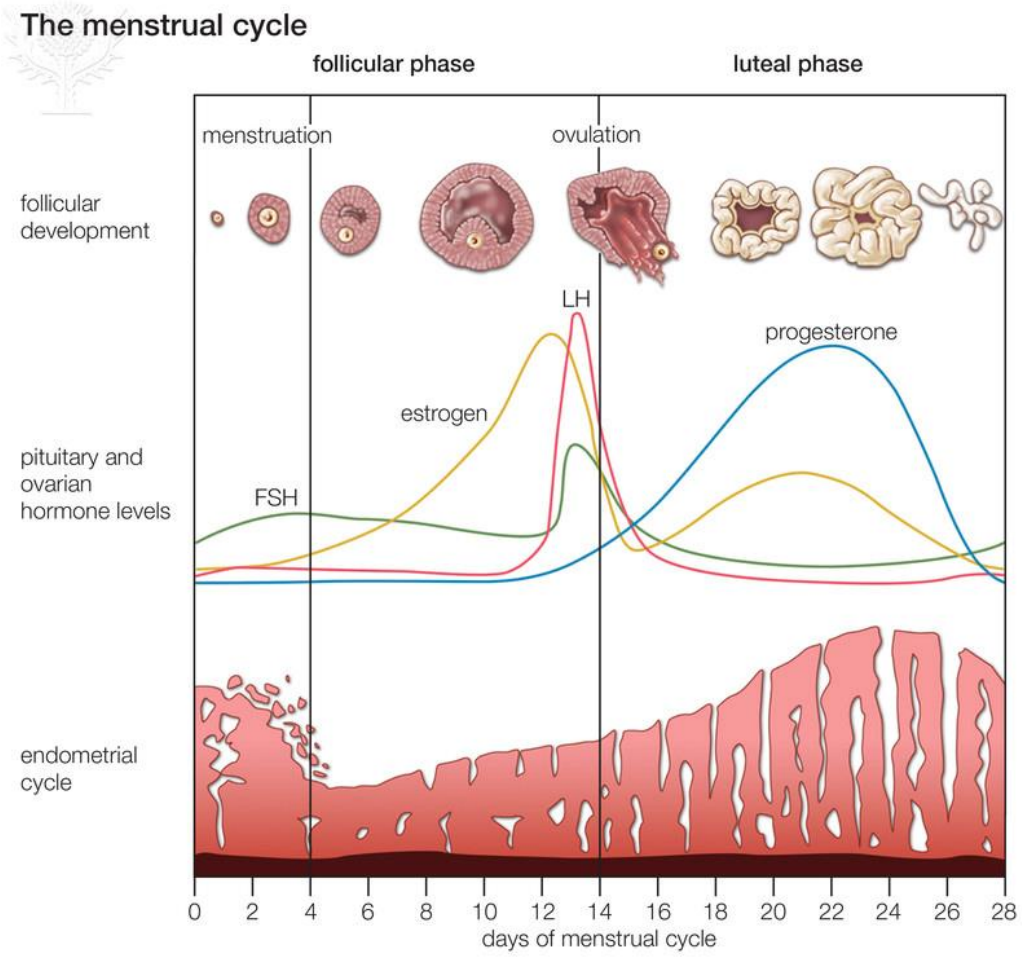
Figure 2.1 The female hypothalamic-pituitary-gonadal axis (12). Feedback to the upper levels of the axis may be negative or positive, i.e. inhibit or stimulate hormone secretion, respectively.



The ovaries contain a population of immature primordial follicles, each consisting of an oocyte surrounded by a layer of granulosa cells which act as supporting cells. The ovarian reserve, which is constituted by the primordial follicle pool, is established during fetal life and steadily declines until menopause through programmed cell death (apoptosis). Starting in puberty, primordial follicles continuously develop into primary, secondary and tertiary (also called antral) follicles, independently of pituitary hormones. At the tertiary or antral stage, the follicles acquire receptors for FSH and can respond to pituitary hormones.

In the early follicular phase of the menstrual cycle (Figure 2.2), the secretion of FSH by the pituitary gland stimulates the growth and development of a group of antral follicles in the ovaries. Eventually, one of the recruited follicles will become selected and dominant while the remaining undergo regression and will die. Follicle selection and dominance are mainly

Figure 2.2 The menstrual cycle (13).



regulated by the high estrogen secretion from the granulosa cells of the dominant follicle, which induces a negative feedback loop causing the FSH levels to decrease. Hence, the stimulation of additional follicles in inhibited. When the dominant follicle reaches a large and critical size, its sustained and high estrogen secretion also leads to a surge in LH inducing final maturation and ovulation. During follicle growth and dominance, a rapid proliferation of the endometrium occurs in response to the estrogen secretion.

The luteal phase is initiated after ovulation, in the middle of the cycle (around day 14 of 28). In the early luteal phase, the granulosa cells of the ruptured follicle transform into a structure called the corpus luteum which produces progesterone and estrogen, causing further proliferation and thickening of the endometrium to make it receptive for implantation. The mature oocyte enters the fallopian tube where fertilization can take place in the presence of spermatozoa. Pregnancy occurs if a resulting embryo is successfully implanted into the receptive endometrium. After implantation, the developing placenta produces human chorionic gonadotropin (hCG) which stimulates continued progesterone production from the corpus luteum and sustains the endometrium. If implantation does not occur, the corpus luteum regresses completely approximately two weeks after ovulation. The resulting drop in progesterone and estrogen causes the menstrual bleeding and releases the hypothalamus from the negative feedback, which in turn causes FSH secretion to increase and a new menstrual cycle to begin.

2.2 FEMALE INFERTILITY

The World Health Organization (WHO) has defined infertility as "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse" (14). This means that it is still possible for an infertile couple to conceive spontaneously, in contrast to sterility. In this thesis, the term infertility generally refers to the WHO definition, which is commonly used clinically and in epidemiological research. There are several other definitions used depending on research field, study question and data available (1).

Among heterosexual couples who seek medical treatment for infertility, female factor infertility alone accounts for 20-35% of cases, male factor infertility explains 20-30% of cases and 25-40% of cases involve both partners. In 10-25% of cases, no cause can be identified and this is defined as unexplained infertility (2,7,15,16).

Fertility is strongly linked to the woman's age, mainly due to the natural depletion of the ovarian reserve and decreased oocyte quality with aging. This age-related decline in female fertility has been shown to accelerate after the age of 37, and spontaneous pregnancies in women over 45 years of age are very rare (6,17). In women of reproductive age, the main causes of infertility are ovulatory dysfunction, structural abnormalities and endometriosis (7,18).

Ovulatory dysfunction includes both anovulation and oligoovulation, i.e. missing or infrequent ovulation. The most common cause of ovulatory dysfunction is polycystic ovary syndrome (PCOS), an endocrine disorder characterized by high androgen levels, polycystic ovaries and menstrual disturbances due to oligo- or anovulation. PCOS is often associated with insulin resistance and obesity, which can negatively affect oocyte maturation, fertilization and implantation. Ovulatory dysfunction can also be caused by premature ovarian insufficiency and disorders of the hypothalamus or pituitary (19).

Structural causes of infertility encompass tubal and uterine factors, with tubal factors being the most common. Tubal damage is most often caused by pelvic inflammatory disease following infections. Pelvic surgery and severe endometriosis can also damage the fallopian tubes. Uterine causes of infertility include congenital malformations of the uterus and acquired conditions such as benign uterine tumors (20).

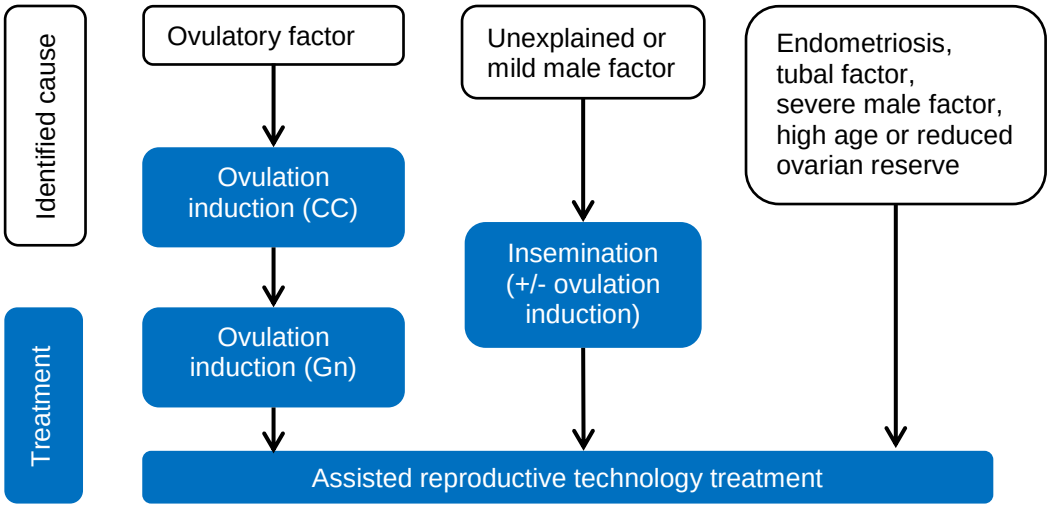
Endometriosis is an inflammatory disease characterized by endometrial tissue growing outside the uterine cavity, commonly associated with chronic pelvic pain and infertility. Previous studies have estimated that endometriosis affects 5-15% of all women of reproductive age, and 25-40% of infertile women. Endometriosis has been suggested to interfere with fertility through pelvic adhesions, ovulatory dysfunction, impaired fertilization and implantation, embryo toxicity, and sperm phagocytosis, although the exact mechanisms are not yet known (20,21).

2.3 FERTILITY TREATMENTS

The main aim of hormonal fertility treatments is to stimulate ovulation or superovulation by acting on the hypothalamic-pituitary-gonadal axis. Ovulation induction refers to treatments that increase the chances of spontaneous conception by restoring normal ovulatory cycles, while ART refers to fertility treatments where both oocytes and sperm are handled in vitro (22). In this thesis, ovarian stimulation refers to both ovulation induction and ART.

Fertility treatments are provided within the Swedish tax-funded healthcare system and treatments are individualized based on the identified causes of infertility (6). The established clinical practice (Figure 2.3) includes ovulation induction using clomiphene citrate as the first-line treatment for anovulatory infertility if no additional infertility factors are present. If unsuccessful, low-dose gonadotropin stimulation may be used to obtain ovulation and subsequently ART is used as a final line treatment. The first choice for mild male factor or unexplained infertility is intrauterine insemination with or without ovulation induction. For infertility due to endometriosis, severe male factor or tubal factor, ART is recommended as the first-line treatment. Since age has a large impact on female fertility, women older than 36 years of age or who present with a strongly reduced ovarian reserve are preferably treated directly with ART to increase the chances of success. Surgical treatment is occasionally required for severe endometriosis, malformations and certain tubal or uterine factors (6).

Figure 2.3 Treatment algorithm for infertility (CC clomiphene citrate, Gn gonadotropins).



2.3.1 Ovulation induction

Ovulation induction treatments encompass hormonal medications used to restore the natural ovulatory cycle in women with irregular cycles and anovulation. Experimental treatments to induce ovulation in humans were performed as early as the 1930s, using gonadotropins from pig, sheep and mare. Though a number of pregnancies were reported, these preparations also triggered the formation of antibodies and the treatments were eventually stopped due to safety concerns. The first reports of pregnancies following human gonadotropin stimulation were published in 1962. The early gonadotropin preparations contained both FSH and LH which

limited their efficiency, and methods to purify FSH from human menopausal gonadotropin were developed over the next decades. Preparations of pure FSH produced using recombinant gene technology became available in the early 1990s (23).

Clomiphene citrate belongs to a group of anti-estrogens first synthesized in the late 1950s. When these drugs were tested in patients with endometrial hyperplasia, they were found to restore menstruation in women with PCOS (5). Following this surprising discovery, clomiphene citrate was approved for the treatment of anovulatory infertility in 1962 in the United States and is still the most commonly used fertility drug worldwide. Clomiphene citrate is known to inhibit the negative feedback of estrogen on the hypothalamus which leads to increased secretion of FSH from the pituitary gland (5).

In Sweden, both clomiphene citrate and gonadotropins have been used for ovulation induction since the 1960s (24). Ovulation induction is usually followed by an injection of hCG, called a trigger shot, which mimics the natural LH surge to trigger the final maturation of the oocyte and ovulation (6).

2.3.2 Assisted reproductive technology (ART)

ART treatments generally encompass a step-by-step procedure including controlled ovarian stimulation (COS) for the retrieval of oocytes by transvaginal and ultrasound-guided ovarian puncture, the fertilization of oocytes with sperm in a laboratory to produce embryos which are subsequently cultured during a few days before being transferred into the woman's uterus. In COS, high and sustained doses of gonadotropins are injected to eliminate the selection mechanism and support the growth of multiple follicles during a single cycle.

The first live birth following ART treatment was reported in 1978 (25). This groundbreaking achievement was accomplished within a natural cycle, where laparoscopic extraction of the single mature oocyte had to be carefully timed with the woman's ovulation. Reports of other successful ART soon followed. The procedures were further developed and a few years later, COS using either clomiphene citrate or gonadotropins replaced natural-cycle ART. In around 20% of the early treatments with COS, the high levels of estrogen induced an early LH surge, causing ovulation before the mature oocytes could be extracted. In order to avoid this, suppression of GnRH was introduced to the treatment protocol in the 1980s (25).

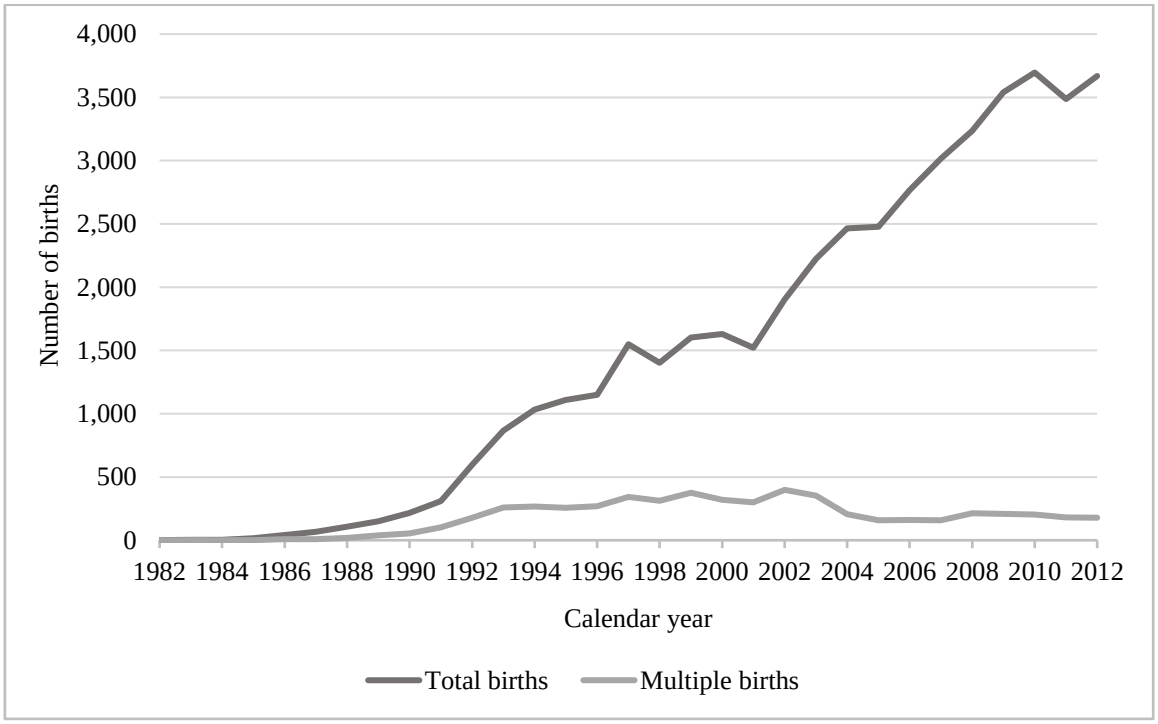
The modern ART procedure follows several steps. Gonadotropins for COS are normally administered in the early follicular phase of the ovulatory cycle. COS has been performed with gonadotropins alone in Sweden since the mid-1990s, before which both clomiphene citrate and gonadotropins were used (26). The most commonly used gonadotropin for ART in Sweden is recombinant FSH.

Prevention of spontaneous ovulation can be achieved by either GnRH agonists or antagonists, depending on the protocol used. In the long protocol, administration of a GnRH agonist is often initiated in the luteal phase of the cycle previous to the treatment. GnRH agonists stimulate the pituitary initially triggering an increase of FSH and LH, called a flare.

Continued GnRH agonist administration leads to downregulation of the gonadotropin receptors on the pituitary and decreased levels of FSH and LH. GnRH antagonists have also been developed. They have a similar effect, suppressing GnRH action on the pituitary immediately and completely, causing decreased FSH and LH levels without the initial flare. In the short protocol, administration of GnRH antagonists is initiated a few days after COS has been started.

After 10-12 days of stimulation, a single injection of hCG is given to trigger the final stage of oocyte maturation. The mature oocytes are collected from the follicles using an ultrasound-guided needle just before they would have been released into the fallopian tubes. In standard *in vitro* fertilization (IVF) sperm are added to the oocytes in a Petri dish. A single sperm can also be injected directly into the center of the oocyte in a procedure called intracytoplasmic sperm injection (ICSI). Normally, one or two of the resulting embryos are transferred to the uterus using a soft catheter, while additional embryos can be frozen for later attempts. Since the ART procedures lower endogenous LH levels, luteal support with progesterone is needed to support implantation and early embryogenesis (6).

Figure 2.4 Total number of births and multiple births following ART in Sweden 1982–2012. Based on data from the National Board of Health and Welfare and the Q-IVF registry.



During the 1980s, the total number of ART births in Sweden were less than 400 (Figure 2.4). Since the 1990s, the number of treatments has increased dramatically. In 2012, there were more than 3,500 deliveries following ART, accounting for 3.8% of all births in Sweden in 2012 (27). The multiple birth rate following ART has historically been very high. In Sweden, a gradual shift to double and single embryo transfers successfully decreased the multiple birth rate from 32% in 1991 to under 5% in recent years (28,29). Oocyte donation was legalized in Sweden in 2003, and accounted for less than 100 (3%) of the ART births in 2012 (29).

2.4 CANCER, INFERTILITY AND FERTILITY TREATMENTS

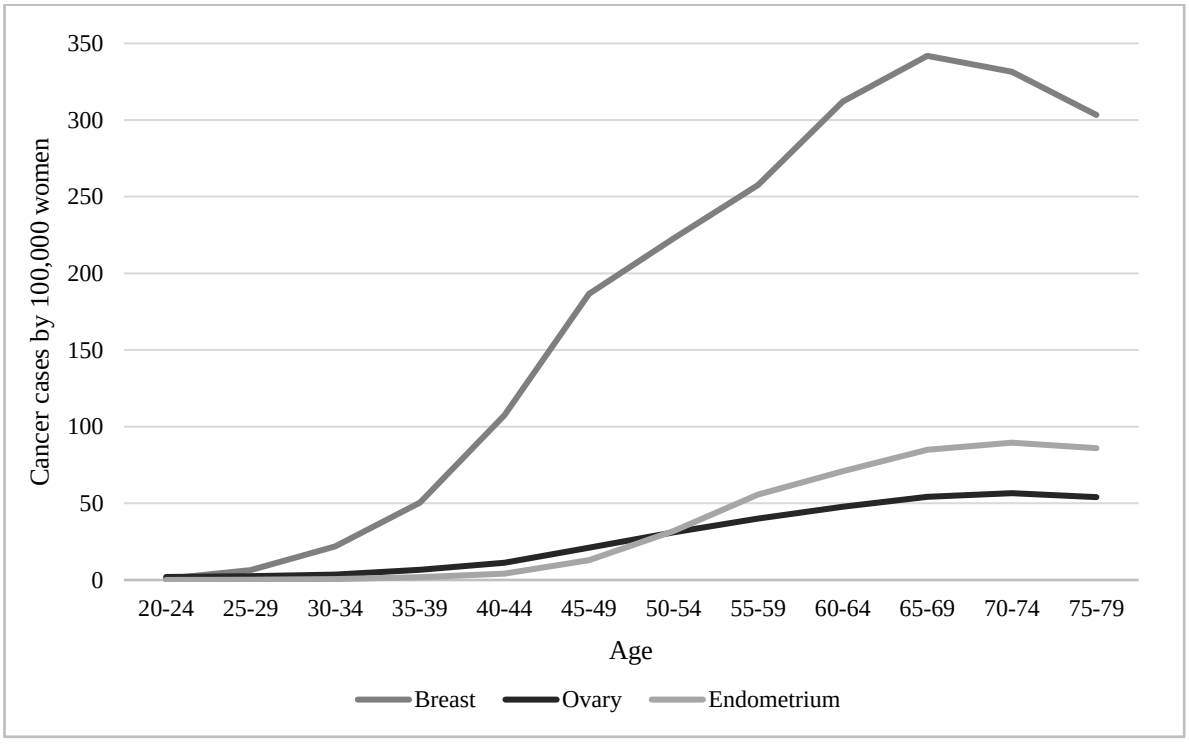
Cancers are broadly classified according to their anatomical location and by the tissue or cell type where they originated. Different cell types give rise to cancers that differ in appearance, behavior and prognosis. Etiology and risk factors for different cancers also vary considerably. Carcinomas, cancers of epithelial origin, account for 90% of all cancer cases (30). The characteristics and risk factors described in this chapter are based on studies where the majority of cancers were carcinomas, and do not necessarily apply to cancers that arise from other cell types such as connective tissue or germ cells.

This thesis project has focused on cancers of the breast, ovary and endometrium. These cancer types are commonly associated with hormonal and reproductive factors and could therefore potentially be influenced by infertility or fertility treatments (31–33).

There are several challenges when investigating cancer risk following fertility treatments. The underlying causes of infertility, reproductive factors such as not having children or giving birth at a later age, and ovarian stimulation treatments often occur together making it difficult to disentangle the potential effect of treatment from that of infertility per se. Cancer is also a relatively rare outcome, which is usually diagnosed decades after the reproductive period, while most women who have received ovarian stimulation so far are still young.

Increasing age is the most important risk factor for the cancers covered in this thesis. Figure 2.5 shows the crude incidence rates of these cancers in Sweden by age group. Breast cancer incidence starts increasing before age 30, while ovarian and endometrial cancer are generally diagnosed in older women. Over the last decades, the incidence of breast cancer has increased while both ovarian and endometrial cancers have become less common (30).

Figure 2.5 Incidence rates of breast, ovarian and endometrial cancer in Sweden 1982–2012, by age (34).



2.4.1 Breast cancer

Breast cancer is the most common cancer type in women, representing 27% of all cancer cases among women in Sweden (35). Not having children, early menarche, late menopause, higher age at first birth and not breastfeeding are established risk factors for breast cancer (36,37). A temporary increase in breast cancer risk has been shown for current or recent use of oral contraceptives at reproductive age or hormone replacement therapy in postmenopausal years (31). Some studies have also suggested associations between breast cancer risk and infertility (38–40), endometriosis (41,42), and PCOS (43,44).

Both clomiphene citrate and gonadotropins have been suspected to influence the risk of breast cancer through increased serum concentrations of endogenous estrogen (9). Reassuringly, most previous studies and meta-analyses have not shown an increased risk of breast cancer following ovarian stimulation (38,45–52). Another recent meta-analysis found no association between ART and breast cancer, but indicated a small increase in risk following non-ART treatments of infertility (9). Additionally, a large cohort study from Norway reported that women who gave birth following ART had a small but statistically significant increase in breast cancer risk compared to other parous women (53). Some studies have also shown an increased risk of breast cancer in subpopulations of infertile women, including women who remained nulliparous following gonadotropin stimulation (54,55), commenced their ART treatment at a young age (56), started treatment after age 30 (57), underwent four or more cycles of ART (58), and women treated with high-dose clomiphene citrate therapy (24).

Mammographic density

Mammographic density, referring to the amount of radiologically dense fibroglandular tissue in the breast, is a strong marker of breast cancer risk (59,60). Mammographic density is associated with both the risk of developing breast cancer and the risk of not detecting a cancer in the screening program (61). High density is also related to reproductive characteristics, such as low parity and high age at first birth (62). These features suggest that mammographic density is an intermediate phenotype in breast cancer etiology (63), making it an interesting trait to study in relation to ART since most women who have gone through treatment are currently still below the age when breast cancer is usually diagnosed.

Few studies have evaluated breast mammographic features in selected populations of infertile women. A study by Sprague et al. (2008) found that women more recently dispensed a fertility drug had lower density, although there were no associations between fertility drug use and mammographic density overall (64). In two more recent studies, Meggiorini et al. reported higher breast density in women with ovulatory infertility compared to women with other infertility types (65), and in women with primary infertility compared to parous women (66).

2.4.2 Ovarian cancer

Ovarian cancer accounts for 3% of incident cancers but over 5% of cancer-specific mortality among women in Sweden (35). Since early symptoms are few and unspecific, ovarian cancer is often diagnosed at an advanced stage when it is more difficult to treat and cure. The risk of ovarian cancer is higher among women with early menarche, late menopause, nulliparity and endometriosis (67,68). In contrast, oral contraceptive use and high age at first as well as last birth are associated with a lower risk of ovarian cancer (69,70). Around 10-15% of ovarian cancer cases are hereditary (71). Some studies have also found an increased risk of ovarian cancer among women with irregular menstruation (72) and women evaluated for infertility (39,40,73).

Two different theories have been proposed to explain these associations: the incessant ovulation theory and the elevated gonadotropins theory. The first hypothesizes that the damage and repair of the ovarian epithelium during ovulation leads to an increased ovarian cancer risk, while the latter suggests that stimulation of the ovarian epithelium by endogenous gonadotropins causes the risk increase. As ovarian stimulation increases the levels of circulating gonadotropins and stimulates ovulation, both of these theories propose a link between treatment and ovarian cancer risk (74).

Numerous studies have investigated the associations between ovarian stimulation and ovarian cancer risk. Most studies to date have not found an increased risk of ovarian cancer following either ART (46–48,68,75,76) or ovulation induction (38,45,77–80). However, some studies have reported higher risks of ovarian cancer following ART (26,81) and ovulation induction (50,82,83). Only two of these studies included comparisons to untreated infertile women, where one reported a higher risk among women treated with clomiphene citrate who remained nulliparous (82), and the other a higher risk following stimulation with gonadotropins alone (83). Consequently, it remains unclear whether any ovarian stimulation treatments may influence ovarian cancer risk or if the associations were due to the underlying infertility.

Borderline ovarian tumors

Borderline ovarian tumors (BOT) are intermediate between benign and invasive ovarian tumors, accounting for around 15% of primary epithelial ovarian tumors in Sweden. These tumors are more common in younger women and have a better prognosis than ovarian cancer. In Sweden, the incidence of BOT has increased while ovarian cancer incidence has declined since the 1980s (84). Although they are generally considered to be separate diseases, BOT and ovarian cancer share similar risk factors (85,86). The results of some previous studies have also suggested that infertility (86), ART (50,76,87) and ovulation induction (83) may increase the risk of BOT.

2.4.3 Endometrial cancer

Endometrial cancer, originating from the inner lining of the uterus, is the most common gynecological malignancy. In Sweden, endometrial cancer accounts for 5% of incident cases among women (35). Lifestyle factors such as obesity, diabetes mellitus and hypertension are known risk factors for endometrial cancer. Nulliparity, late menopause and PCOS also increase the risk, while oral contraceptive use, physical activity, cigarette smoking, increased parity, and giving birth late in life seem to be protective. Hormone replacement therapy using only estrogen has also been shown to increase the risk of endometrial cancer, while combination therapy with estrogen and gestagens do not (33,88). Many of the identified risk factors are associated with increased levels of estrogen without opposing progesterone, a situation that has been consistently found to elevate endometrial cancer risk (88).

Several studies have reported a higher risk of endometrial cancer among infertile women (38,40,45,89). The associations may be due to PCOS, although these studies did not have information on the causes of infertility. Some studies have also suggested that endometriosis could increase the risk of endometrial cancer (41,90), while others have not (42,91,92).

A recent systematic review found a higher risk of endometrial cancer following ovarian stimulation, in particular for multiple cycles of clomiphene citrate, although the authors noted that the association could still be due to the underlying infertility (93).

3 AIMS

The overarching aim of the thesis project was to perform a large population-based assessment of whether infertility or the use of assisted reproductive technology impacts cancer risk.

The specific research questions for each study in this thesis were:

- **Study I:** Does ovarian stimulation alter mammographic density?
- **Study II:** Does ovarian stimulation affect the risk of breast cancer?
- **Study III:** Do ART treatments influence the risk of invasive ovarian cancer or borderline ovarian tumors in parous women?
- **Study IV:** Is infertility associated with the risk of breast, ovarian or endometrial cancer and, if so, can ovulatory disturbances or endometriosis explain the association?

4 MATERIALS AND METHODS

4.1 DATA SOURCES

Information from Swedish national population-based registers and quality registers was used in all four studies. The unique personal identification numbers assigned to all Swedish residents enables individual record linkage (linking) of these registers. Studies II-IV were based entirely on data from the national registers while study I also used data from a prospective breast cancer cohort called Karma.

4.1.1 The Multi-Generation Register (MGR)

The Swedish Multi-Generation Register (MGR), held at Statistics Sweden (Statistiska Centralbyrån), is a register of individuals born 1932 or later and residing in Sweden at any time since 1961 (94). The MGR links these so-called index persons to their biological and adoptive parents. Statistics Sweden created the MGR in the year 2000 using information from the population registry held by the Swedish Tax Agency and updates it yearly. Parents of index persons must have resided in Sweden in 1947 or later to be included in the MGR. Information on parents is also missing for around 40% of the index persons who died before July 1991, when the church was responsible for administration of the population registry.

4.1.2 The Medical Birth Register (MBR)

The Medical Birth Register (MBR) was started in 1973 by the National Board of Health and Welfare (NBHW, Socialstyrelsen). The MBR contains detailed information on all childbirths in Sweden, obtained from medical records of prenatal, delivery and neonatal care (28). For the purpose of monitoring children born through ART treatments, information on ART treatments leading to a live birth was retrospectively collected from IVF clinics by the NBHW for the years 1982–2006 and linked to the MBR (28,95). This information includes the date of embryo transfer, ART method used and the personal identification number of mothers and children.

4.1.3 The National Quality Registry for Assisted Reproductive Technology (Q-IVF)

The National Quality Registry for Assisted Reproductive Technology (Q-IVF) covers ART-treatments performed in Sweden since 2007. All ART cycles are recorded in the registry, including those that do not lead to pregnancy or live birth. The registry is administered jointly by all ART clinics in Sweden and contains detailed information about each cycle of ART, including stimulation protocol, oocytes and sperm used, fertilization method, number of embryos transferred, treatment results and the date of each treatment step. The personal identification number for both parents is also recorded.

4.1.4 The National Patient Register (NPR)

The National Patient Register (NPR), held at the NBHW, was established in 1964. During the first year, somatic in-patient care was collected from six Swedish counties as a pilot project. Gradually, more counties joined the project and the NPR covered 85% of all hospital discharges in 1983. Since 1987, the NPR has complete national coverage of in-patient care. Registration of surgical procedures in out-patient care was introduced in 1997 and expanded in 2001 to include all specialist out-patient care, with an estimated completeness of around 80% (96,97).

The records in the NPR include the personal identification number of the patient, date of admission and discharge, primary diagnosis, up to eight additional diagnoses and any surgical procedures performed during the visit. Each diagnosis is coded using the current version of the International Classification of Disease (ICD). ICD version 7 was used until 1968, ICD-8 1969–1986, ICD-9 1987–1996, and ICD-10 from 1997. Skåne County switched to ICD-10 in 1998. Surgical procedures were coded using a national classification system in 1964–1996 (98) and changed to a Nordic system in 1997 (99). Consequently, both the codes for diagnoses and surgical procedures have changed during the period covered in the included studies.

4.1.5 The Prescribed Drug Register (PDR)

The Prescribed Drug Register (PDR), held at the NBHW, includes every dispensation of prescription drugs at Swedish pharmacies since July 2005. The PDR is automatically updated every month (100). In addition to the personal identification number of the patient, each entry in the register contains information on date of prescription and dispensation, the brand name of the prescribed drug and its active substance coded using the Anatomical Therapeutical Chemical (ATC) classification system from the WHO. The PDR does not contain information on vaccines or drugs administered in hospital in-patient care.

4.1.6 The Swedish Cancer Register (SCR)

The Swedish Cancer Register (SCR) records all occurrences of primary malignant tumors, as well as certain primary non-malignant tumors, in Swedish residents since 1958. Each entry includes date of diagnosis, tumor site, clinical and morphological diagnosis, as well as information about the patient and the reporting hospital. The tumor location is coded using the current version of the International Classification of Diseases for Oncology (ICD-O), and tumor morphology according to ICD-O and the Systematized Nomenclature of Medicine (Snomed). Table 4.1 describes when new coding systems and versions were added to the SCR (101). Both location and morphology codes are translated to previously used coding systems, so that all tumors registered in the SCR since 1958 have an ICD-7 location code and morphology coded using a historical three-digit system (C24) developed by the WHO in 1956 (102). The SCR has an estimated completeness of over 96% for gynecological cancers and 98% for breast cancers (103).

Table 4.1 Coding systems used for tumors in the SCR.

Year	Location	Morphology
1958	ICD-7	C24
1987	ICD-9	
1993	ICD-O/2	Snomed (ICD-O/2)
2005	ICD-O/3	Snomed (ICD-O/3)

4.1.7 Other registers

The Education Register includes the current highest educational level, place of education and personal identification number for Swedish residents. Statistics Sweden administers the register which contains information reported from all schools and universities since 1985.

Migrations in and out of Sweden are recorded in the Total Population Register, held at Statistics Sweden. Emigrations out of Sweden have been included since 1961, and immigrations since 1969. The available data includes the date and direction of each migration as well as the personal identification numbers.

Information on date and cause of death for Swedish residents is recorded in the Cause of Death Register since 1961. There is also a historical register covering the years 1952–1960, and both of these registers are held at the NBHW.

4.1.8 The Karma cohort

Study I was based on data from the Karolinska Mammography Project for Risk Prediction of Breast Cancer, Karma, which is a screening-based breast cancer cohort (104). Between 2010 and 2013, more than 70,000 women were recruited to Karma from four participating mammography units in Stockholm County and Skåne County. In Sweden, all women aged 40 to 74 years are invited to mammography screening at an interval of 18 to 24 months. The age groups which are offered screening have varied over time, and ages 70 to 74 were not included in the Stockholm screening program until 2013 (105). Detailed information on the women's background and reproductive health, including menstruation history, fertility problems and treatments, medication, alcohol and tobacco use, chronic diseases, life-style factors and family history of cancer was collected in a comprehensive self-report questionnaire at the first screening visit. Digital mammographic images are stored after each screening visit. Information from national registers, including the NPR, SCR, PDR and the Cause of Death Register, is linked to the cohort and updated every six months.

4.1.9 Register linkage for studies II-IV

Studies II-IV were based on data from Statistics Sweden, the NBHW and the Q-IVF registry. The index population consisted of all women born between 1932 and 2012 and registered as index persons in the MGR at any time from 1961 until 2012. Statistics Sweden identified the population and created a key file linking the personal identification numbers to randomly

assigned sequence numbers. The personal identification number was replaced with these sequence numbers in all data delivered for research.

Information on ART has been retrieved from the NBHW (1982–2006), from the PDR (July 2005–2012) and from the Q-IVF registry for the years 2007–2012. For the years 1982–2006 only information on ART treatments leading to a live birth was available, while the Q-IVF registry contains information on all ART cycles (including unsuccessful treatments). The PDR was used to find information on dispensations of ovulation-stimulating drugs since 2005 and onwards. Medical information including diagnoses of infertility and cancer was also obtained from the registers held at the NBHW. Socioeconomic data such as education, country of birth and migrations was provided by Statistics Sweden.

4.1.10 Ethical considerations

Research conducted in Sweden that involves individual human beings is regulated by the Ethical Review Act (2003:460), The Public Access and Secrecy Act (2009:400) and the Personal Data Act (1998:204). The regulations protect study participants against the risk of physical injury, mental injury or the violation of integrity. Research that includes the handling of sensitive personal data may only be conducted following an ethical vetting process which provides an approval. The ethical vetting process includes weighing the risks or harms involved against the potential gain of knowledge by the proposed research.

Study I used data from the Karma cohort. Ethical permission for the Karma project was obtained in 2010 (2010/958-31/1). The ethical approval was updated in 2014 in order to link ART information to the cohort (2014/11-32). More than 70,000 women attending mammography screening between 2010 and 2013 were recruited to Karma. All women who chose to participate in the study provided written informed consent. They were also informed that they can withdraw from the study at any time (page 19-24 in the ethical application form). In the datasets used for the studies in this thesis, personal identification numbers were replaced by unique study participant numbers and other personal identifiers were removed before the research team gained access to the data.

Ethical approval for the register linkage used in studies II-IV was granted in 2013 (2013/1849-31/2) and updated in 2014 (2014/118-32). All of the information used in these studies was obtained from registers at central authorities and national quality registers in Sweden and the project, including eleven million individuals, was granted an exception to the requirement of informed consent. The personal identification numbers have been replaced by randomly generated unique sequence numbers and the key is held at the NBHW for future updates. The research team did not have access to the original identification numbers but only the random sequence numbers.

All of the four studies involved handling of large amounts of sensitive personal information. While the files do not contain any names, addresses or personal identification numbers, it would still be possible to identify specific individuals through other information such as their date of birth and medical history. However, as researchers we are obliged by law to protect

the privacy of the participants and not misuse any information discovered. The data is handled and stored in a secure way to minimize the risk of data misuse, and the results are presented in an aggregated form so that identification of individual participants is not possible.

These and other similar studies contribute important knowledge about the effects of infertility and fertility treatments on human health. The results may be used to improve diagnosis and treatment of infertility and might also identify risk factors for cancer development.

4.2 MEASUREMENTS, ASCERTAINMENT AND DATA QUALITY

4.2.1 Infertility

In study I, women with a history of infertility were identified using self-reports. The Karma questionnaire included the question "Have you ever tried to become pregnant for one year or more without success?" corresponding to the WHO definition of infertility.

Table 4.2 Infertility-related diagnoses in the NPR.

Diagnosis	ICD-7 1964–68	ICD-8 1969–86	ICD-9 1987–96	ICD-10 1997–
Female infertility	636	628	628	N97
Due to anovulation	n/a	n/a	628A	N97.0
Due to endometriosis	n/a	n/a	n/a	N97.8D
Endometriosis	n/a	625,3	617	N80
Ovarian dysfunction	275	256	256	E28
Absent, scanty or rare menstruation	634,10-634,12	626,00-626,11	626A-626B	N91.1-N91.5

n/a indicates that the diagnosis was not available in the current version of ICD.

The diagnosis codes listed in Table 4.2 were used to define a comparison group of women with fertility-related problems in study II. A stricter definition, including only women diagnosed with infertility, was used to identify a comparison group in study III. In study IV, the diagnoses in Table 4.2 were used to subdivide women with and without infertility diagnosis into three groups having ovulatory disturbances, endometriosis, or neither. Infertility was defined as any diagnosis of female infertility, ovulatory disturbances as any diagnosis of ovarian dysfunction, menstrual disturbances, or infertility due to anovulation, and endometriosis as any diagnosis of endometriosis or infertility due to endometriosis. The definitions used in each study are summarized in Table 4.3.

Table 4.3 Definitions of infertility and related diagnoses used in study II-IV.

Infertility-related diagnosis Study II	Infertility Studies III and IV	Ovulatory disturbances Study IV	Endometriosis Study IV
• Female infertility of any cause • Endometriosis • Ovarian dysfunction • Absent, scanty or rare menstruation	• Female infertility of any cause	• Ovarian dysfunction • Absent, scanty or rare menstruation • Female infertility due to anovulation	• Endometriosis • Female infertility due to endometriosis

4.2.2 Ovulation induction and controlled ovarian stimulation

In the Karma questionnaire, women reporting a history of infertility were also asked if they had ever received fertility treatment and if so, which of the following: hormonal treatment only, sperm insemination, IVF/ICSI, IVF with egg donation, surgical treatment, and other treatment. Women answering 'other treatment' were asked to describe the treatment in an open field. In study I, the answers were used to define three treatment categories: COS, ovulation induction, and no hormonal treatment. The COS category consisted of women who had ever been treated with COS for ART (IVF or ICSI). IVF with egg donation was categorized as non-hormonal since women receiving donated eggs do not require COS. Women who had answered 'hormonal treatment only', or described using either clomiphene citrate (Pergotime, Clomivid) or cyclofenil (Sexovid) in the open field were included in the ovulation induction category. The majority of women in the category 'no hormonal treatment' had not received any fertility treatment, while a small fraction had gone through non-hormonal treatments including surgery and insemination.

In study II, the PDR was used to identify women who had gone through ovulation induction or COS for ART. COS was defined as dispensations of gonadotropins and GnRH analogues (either agonist or antagonist). In the short COS protocol, GnRH antagonist treatment is normally initiated a few days into the stimulation and prescribed at the same time, while down-regulation with GnRH agonists is initiated 10-14 days prior to stimulation in the long protocol. Up to 90 days was allowed between dispensations of down-regulation and stimulation, in case the GnRH analogue had been dispensed for a previous treatment cycle. Ovulation induction was defined as dispensations of either clomiphene citrate or gonadotropins without down-regulation. Trigger shots with hCG were not included in the algorithms since they are used in both COS and ovulation induction. The names and ATC-codes of included fertility drugs are listed in Table 4.4.

Table 4.4 Prescribed drugs used to treat infertility with corresponding ATC-codes.

Ovarian stimulation	Down-regulation
clomiphene citrate, G03GB02	*GnRH agonists*
Gonadotropins	nafarelin, H01CA02
menotropin, G03GA02	buserelin, L02AE01
urofollitropin, G03GA04	triptorelin, L02AE04
follitropin alfa, G03GA05	*GnRH antagonists*
follitropin beta, G03GA06	ganirelix, H01CC01
chorifollitropin alfa, G03GA09	cetrorelix, H01CC02

4.2.3 ART births

In studies II and III, information from the NBHW and the Q-IVF registry were used to identify women with ART births 1982–2012. ART births between 1982 and 2006 were identified by linking the NBHW information to the MGR, using the unique sequence numbers of each mother and child. For the later years, information on embryo transfer date and delivery date in the Q-IVF registry was used. Since the Q-IVF registry does not record

the personal identification number of the children, it was linked to the MGR using the sequence number of the mother and the birthdate of the child. When the birthdate of the child did not match, a birth in the MGR within ten months of an embryo transfer in the Q-IVF registry was categorized as an ART birth. Both fresh and frozen cycles from standard IVF and ICSI were included. Additionally, ART using donated oocytes were included since information on oocyte origin was missing for the years 2003–2006.

4.2.4 Mammographic density

In study I, associations between infertility, fertility treatments and mammographic density were examined. Volumetric measures of density were obtained from digital mammograms using a fully automated method (106). The method computes the volume of dense fibroglandular tissue and the total breast volume. Percent dense volume is the ratio between the absolute dense volume and the total breast volume. The absolute non-dense volume, or amount of fatty tissue, was calculated as the difference between the total and dense volume. Both absolute and relative dense volume have been shown to be associated with breast cancer risk (61,107). For completeness, analyses of all three measures were included in the study.

4.2.5 Cancer

Incident cancers were identified using information from the SCR. The first occurrence of cancer for each woman was included in the studies II, III and IV, and the date of diagnosis was used to identify cases or for censoring follow-up time.

In study II, breast cancer was defined as adenocarcinoma of the breast using ICD-7 code 170 and C24 code 096. This definition was used to identify cancer cases as well as history of breast cancer in first-degree family members (biological mothers and sisters).

In study III, ovarian cancer was defined as any malignant tumor of the ovary, fallopian tubes, broad ligaments (ICD-7 175) or peritoneum (ICD-7 158). Tumors at these sites have similar characteristics and treatment (71). Epithelial tumors were identified using the C24 code 096. Borderline malignant ovarian tumors were defined as tumors with ICD-7 code 175 and C24 code 094. Separate analyses were performed for BOT and ovarian cancer, and women diagnosed first with BOT and later with ovarian cancer were included as cases in both analyses.

In study IV, cancers of the breast, ovary (including fallopian tubes and broad ligaments) and endometrium (ICD-7 170, 175 and 172, respectively) were included. Separate analyses were performed for each cancer type, and women diagnosed with multiple primary malignant tumors at the same date were included as cases for each cancer type.

Women diagnosed with any malignant tumor (ICD-7 140-105) before each study were excluded. In analysis of BOT incidence, women diagnosed with BOT before the study were also excluded.

4.2.6 Other covariates

In study I, the multivariable adjusted model included age at mammography, body mass index (BMI, calculated from height and weight as kg/m^2), cigarette smoking, alcohol consumption, education level, family history of breast cancer, age at menarche, menopausal status, and parity. Breast cancer in first-degree relatives (mothers, sisters and daughters) was identified using the SCR, while the other covariates were retrieved or calculated from questionnaire data.

All multivariable analyses in studies II-IV were adjusted for age, parity, calendar time, education level and age at first birth. In study II, family history of breast cancer was also included, while family history of either breast or ovarian cancer was included in study III. Bilateral oophorectomy, hysterectomy and salpingectomy were included as covariates or used for censoring depending on the cancer type in studies III and IV.

4.3 STUDY DESIGNS

This thesis includes four observational studies. Study I was a cross-sectional study based on a screening cohort where all of the information was collected at a specific time. The other three studies were population-based cohort studies where information on exposure, outcome and other covariates had been continuously recorded in national registers over the course of the study follow-up.

Study population in study I

All women aged 40 to 69 years with digital mammograms from the first mammography visit were selected from the Karma cohort. Women with cancer or breast surgery before mammography, and women with incomplete covariate information were excluded.

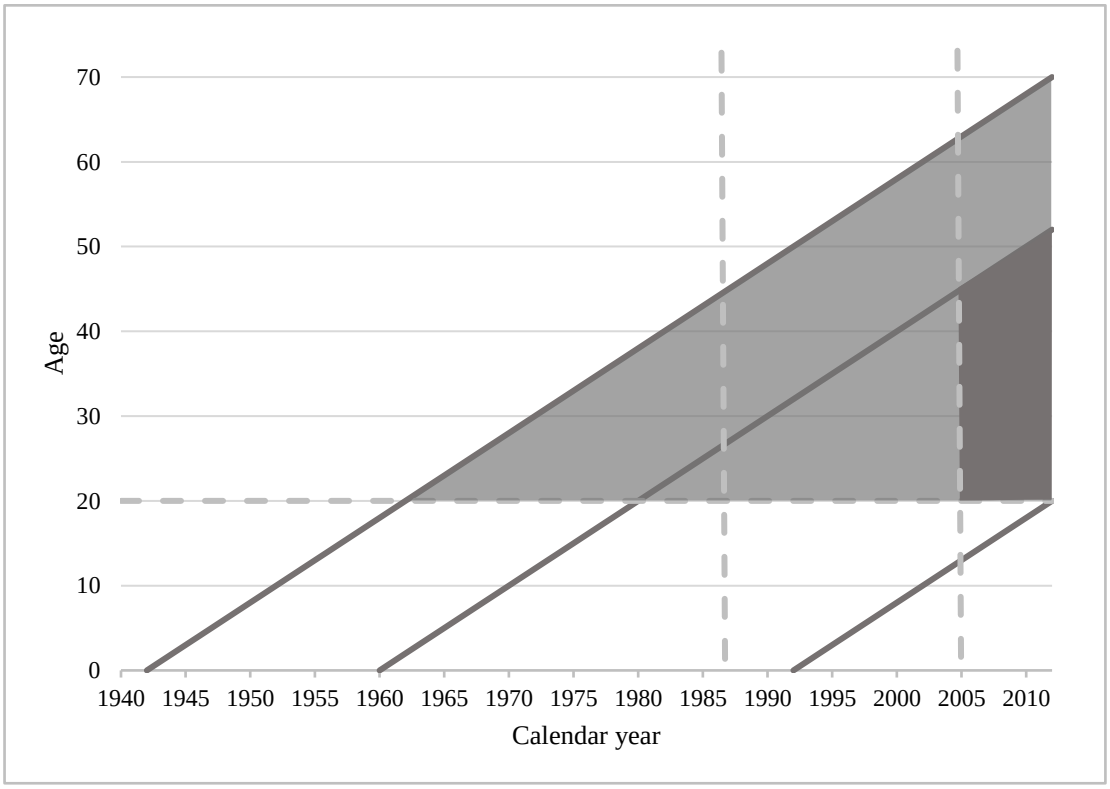
Cohort of parous women in studies II and III

Since only information on ART treatments leading to live births was available for most of the time that ART has been used in Sweden, the study population was restricted to women with live births 1982–2012. Initially, all women who had their first child during this calendar period were identified using information from the MGR. The women were followed from the start of pregnancy resulting in their first child. Pregnancy start was calculated by subtracting gestational length from the birthdate of the child, using gestational length registered in the MBR or 280 days when this information was missing. Women with invalid personal identification numbers, who did not reside in Sweden at the start of follow-up or who had previously been diagnosed with cancer were excluded from the population. In study III women who had bilateral oophorectomy before start of follow-up were also excluded since they were not considered at risk for ovarian tumors.

Birth cohorts in studies II and IV

By using information from the PDR and the Q-IVF registry, all women who had received ovulation induction or COS between 2005 and 2012 were identified. A cohort of women who were between 20 and 45 years old during this time, meaning women born 1960–1992, was established in order to investigate associations between ovarian stimulation treatments and breast cancer risk in study II. The women were followed from their 20th birthday or July 1st 2005, whichever occurred last. Women who were alive and residing in Sweden at the start of follow-up were included, and women with invalid personal identification numbers, who had been diagnosed with cancer or had given birth to four or more children before start of follow-up were excluded. Lexis diagrams, with attained age on the vertical axis and calendar year on the horizontal axis, are useful to visualize birth cohorts. The darker grey area in Figure 4.1 represents the follow-up time for this cohort.

Figure 4.1 Lexis diagram of the birth cohorts in study II and IV.



In study IV, a cohort of women who could have been diagnosed with infertility or related disorders in the NPR was established. Hence, the cohort included women born 1942–1992, who were between 20 and 45 years old in 1987 (when the NPR reached complete national coverage). The women were followed from age 20 and any diagnosis related to infertility from age 15 was included. Both the grey areas in Figure 4.1 represent the follow-up time for this cohort. Women with invalid personal identification numbers, who had died, been diagnosed with cancer or had more than four children before age 20 were excluded.

4.3.1 Bias in observational studies

Observational studies are used to measure associations between exposure and outcome in a population. However, researchers are often interested in whether the exposure actually has a causal effect on the outcome and not just that the two factors tend to appear together. In two theoretical scenarios where the exposure was either present or absent while all other conditions were held constant, the exposure has a causal effect if the two outcomes differ. In epidemiological studies, only one of the scenarios can be observed for each individual, while the scenario that did not occur is called the counterfactual. In order to evaluate whether an observed association may be due to a causal effect, the counterfactual can be approximated by ensuring that all other factors are equally distributed between exposed and unexposed individuals.

In observational studies, unequal distribution of these factors can be caused by systematic or random error. Random error is variation due to chance, which normally decreases with increasing sample size. The main sources of systematic error can be classified into three categories: information bias, selection bias and confounding.

Information bias

Information bias results from inadequate methods of measuring the exposure or outcome variables. Information bias is referred to as measurement error for continuous variables and misclassification for categorical variables. If the rate of misclassification differs depending on exposure or outcome category, called differential misclassification, it can result in over- or underestimation of the effect. Non-differential misclassification generally leads to larger variation in the data, which can cause an underestimation of the effect.

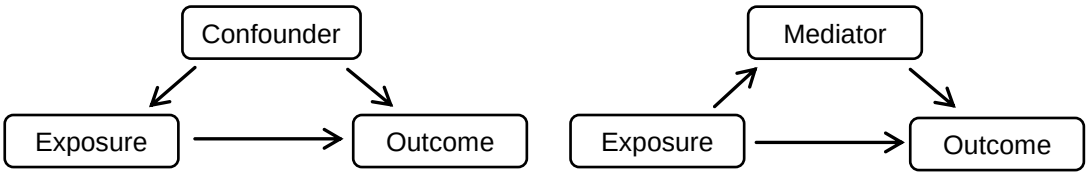
Selection bias

Selection bias occurs if the participants in the study are not representative of the population, in particular with regards to the exposure distribution. In cohort studies, selection bias can be caused by differential loss to follow-up, or if non-responders differ from those who do respond to questionnaires.

Confounding

A confounder is a factor that influences the likelihood of both the exposure and the outcome (Figure 4.2). Confounding can cause bias in both directions (either inflate an estimate or bias it towards the null). In clinical trials, confounding is avoided by randomizing individuals to either receive the exposure or not. In observational studies, confounding can be handled by stratification or by adjustment in regression analyses. A factor that lies in the causal pathway between the exposure and the outcome is called a mediator (Figure 4.2). Adjusting for a mediator blocks this pathway, resulting in a model that can only estimate the direct effect of the exposure on the outcome without mediation and not the total causal effect.

Figure 4.2 Directed Acyclic Graphs demonstrating a confounder (left) and a mediator (right).



4.4 STATISTICAL METHODS

There are many different statistical approaches used in epidemiological research. In general, the methods are used to compare the outcome (e.g. cancer incidence) among exposed and unexposed individuals and to assess the variability in the data to give a measure of how likely it is that the result is due to chance. In this thesis work, two different types of regression models were used to estimate associations with 95% confidence intervals (CI) while adjusting for confounding factors.

4.4.1 Linear regression

Linear regression is normally used for continuous outcomes, such as the mammographic density measures in study I. A simple linear regression model tries to fit observed data of an outcome variable μ and a single explanatory variable x to a linear equation, as shown in Equation 4.1.

$$\mu = \beta_0 + \beta_1 x \qquad \textbf{Equation 4.1}$$

The coefficient β_0 is known as the intercept and can be interpreted as the mean value of the outcome for an unexposed individual, i.e. where x = 0. The regression coefficient β_1 is the mean difference of the outcome μ for a one-unit change of the exposure variable x. The linear regression model can easily be expanded to fit data with more than one explanatory variable x, usually a number of potential confounders x_2 to x_n. This model, called multiple linear regression, is described in Equation 4.2.

$$\mu = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_n x_n \qquad \textbf{Equation 4.2}$$

Linear regression is based on several underlying assumptions about the data. There are a number of extensions of linear regression that allow some of these assumptions to be relaxed. In study I, the associations between infertility, fertility treatment and mammographic density were analyzed using a generalized linear model (GLM) with a log link to account for the skewed outcome distribution (Equation 4.3).

$$\log(\mu) = \beta_0 + \beta_1 x_1 + \cdots + \beta_n x_n \qquad \textbf{Equation 4.3}$$

The GLM yielded an intercept (β_0) equal to the log mean density in the reference group and beta coefficients (β_i) equal to the log mean ratios between exposed and unexposed groups.

Consequently, mean ratios are obtained by exponentiating β_i. In study I, the log mean ratios for the main exposures x_i were transformed to mean differences by subtracting the mean ratio of the unexposed group from that of the exposed group, as explained in Equation 4.4.

$$\textit{Mean difference} = \exp(\beta_0 + \beta_i) - \exp(\beta_0) = \exp(\beta_0) * (\exp(\beta_i) - 1) \quad \textbf{Equation 4.4}$$

The mean difference is interpreted as the difference in mean mammographic density between the exposed and unexposed group on the absolute scale, while the mean ratio is a relative measure interpreted as the ratio of the mean density in the exposed over unexposed group. A mean difference of 0, or a mean ratio of 1, implies that the exposure is not associated with the outcome.

4.4.2 Survival analysis

In cohort studies, the individuals are usually followed for different amounts of time during which they are at risk for the outcome. Regression models that account for different lengths of follow-up time include Cox regression and Poisson regression models. If the outcome does not occur before the end of the study, for example if the individual is lost to follow-up for other reasons, the follow-up time is said to be censored. In studies of cancer incidence, the outcome of interest is a diagnosis of cancer while censoring can be due to other events such as emigration or death.

In studies II-IV, cancer incidence was examined using the Cox proportional hazards model. The hazard function $\lambda(t)$ describes the instantaneous event rate at time t, for those who are alive at time t. In the Cox model, $\lambda(t)$ is modelled as a function of covariates x_i (Equation 4.5), where $\lambda_0(t)$ denotes the baseline hazard function, x_1- x_n are covariates and β_1- β_n their estimated coefficients.

$$\lambda(t) = \lambda_0(t) * \exp(\beta_1 x_1 + \cdots + \beta_n x_n) \quad \textbf{Equation 4.5}$$

The hazard ratio (HR) of covariate x_i is given by the exponent of its coefficient ($\exp(\beta_i)$). For the exposure variable, the HR is the ratio of the hazard in the exposed over the unexposed group, for a certain period of time. When studying cancer incidence, the hazard represents the incidence rate while the HR is an incidence rate ratio. The Cox model estimates these coefficients without making any assumptions about the shape of the baseline hazard.

A strong assumption in the Cox model is that the hazards are proportional over time, meaning that the effects of the covariates on the hazard function are assumed to be multiplicative for the duration of follow-up. The proportional hazards assumption can be formally tested using Schoenfeld residuals to tests the null hypothesis of proportionality. By plotting the hazard functions (e.g. incidence rates) of each exposure category, the proportionality can also be assessed visually.

Since age is a strong risk factor for cancer, attained age was used as the underlying time-scale in all models and the baseline hazard was the incidence rate over age. Hence, all the estimated HRs from the Cox model were adjusted for age. When there is more than one time-line of interest, the follow-up time of each individual can be split into several intervals to control for effects of these timescales. In studies II-IV, the follow-up time was split by calendar period.

Variables that are not constant during follow-up can be entered into the model as time-varying covariates. In studies II and III, fertility treatments were treated as time-varying exposures, as were diagnoses related to infertility in study IV. Parity was included as a time-varying covariate in studies II-IV. In studies III and IV, bilateral oophorectomy, salpingectomy and hysterectomy were also included as time-varying covariates when not used for censoring.

5 RESULTS

The main findings from each study are summarized below. A simplified directed acyclic graph summarizing the study aims is presented in Figure 5.1.

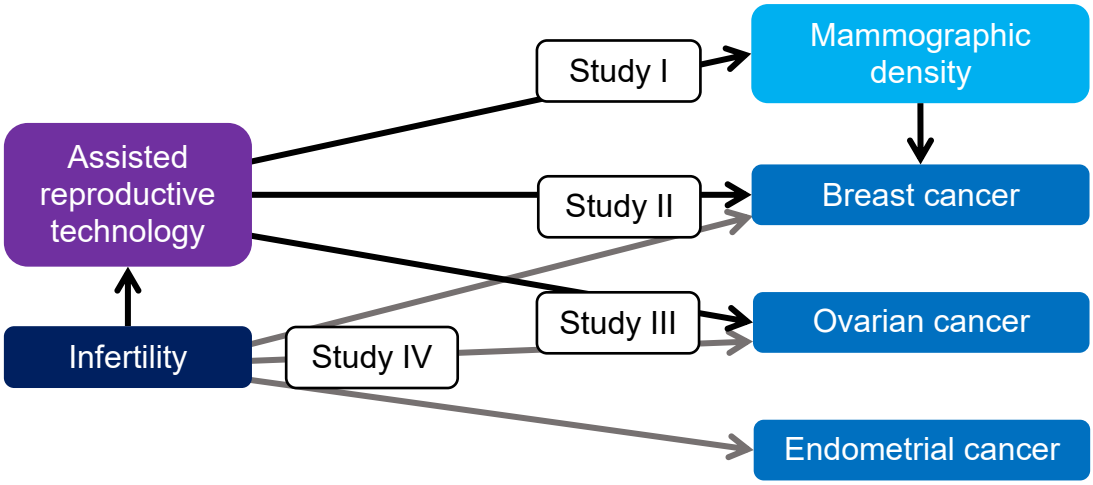
I. Overall, infertile women had a higher absolute dense volume compared to women without fertility problems. Among infertile women, COS was associated with a higher absolute dense volume while other hormonal treatments were not. Percent volumetric density was not associated with infertility or fertility treatments.

II. Women who gave birth following ART did not have a higher incidence of breast cancer compared to women with non-ART births. Neither COS nor ovulation induction treatments were associated with a higher breast cancer incidence, compared to women who did not receive treatment.

III. Compared to women with non-ART births, women who gave birth following ART had a higher incidence of both ovarian cancer and BOT. Compared to infertile women who conceived without ART, women with ART births had a statistically significant higher incidence of ovarian cancer but not BOT.

IV. Women diagnosed with infertility had a higher incidence of ovarian and endometrial cancer, but not of breast cancer. Ovarian cancer incidence was higher in women diagnosed with endometriosis, and in nulliparous women with ovulatory disturbances, compared to women with none of the diagnoses. Endometrial cancer incidence was higher in women with ovulatory disturbances, but not in women with endometriosis.

Figure 5.1 Overview of associations explored in the included studies.



5.1 STUDY I

Study I examined the associations between infertility, hormonal infertility treatments and mammographic density in a cohort of 43,313 women aged 40-69 years at mammography screening. The main results from this study were:

- One fifth of all women reported a history of infertility. Among these women, 66.4% reported no hormonal treatment, 17.6% had gone through COS for ART and another 16.0% had ovulation induction only.
- Among nulliparous women 31.9% had tried to become pregnant, out of which 59.6% had received no hormonal treatment, 28.5% had been treated with ART and another 11.9% with ovulation induction.
- Women with a history of infertility had 1.53 cm^3 higher absolute dense volume (95% CI 0.70–2.35), compared to women with no infertility in the multivariable adjusted analysis. Since non-dense volume was also higher (adjusted mean difference 7.6, 95% CI 1.8–13.4), percent dense volume did not differ between infertile and non-infertile women (adjusted mean difference 0.09, 95% CI -0.06–0.23).
- Compared to infertile women who did not receive hormone treatment, absolute dense volume was higher among infertile women treated with ART (adjusted mean difference 3.22, 95% CI 1.10–5.33), but not among women who had only gone through ovulation induction (adjusted mean difference -0.94, 95% CI -2.77–0.89).
- Percent dense volume did not differ between infertile women who had received either ART (adjusted mean difference 0.19, 95% CI -0.14–0.52) or other hormonal stimulation (adjusted mean difference -0.20, 95% CI -0.54–0.15), compared to infertile women who did not receive hormone treatment.
- The association between absolute dense volume and ART treatment seemed to be stronger among nulliparous women (adjusted mean difference 7.33, 95% CI: 2.29–11.38) than parous women (adjusted mean difference 2.21, 95% CI: -0.02–4.44), although the likelihood ratio test for interaction did not reach statistical significance ($p = 0.073$).

5.2 STUDY II

Study II investigated breast cancer incidence following ovarian stimulation in two separate cohorts. Associations between births following ART and breast cancer incidence were assessed in a cohort of parous women with a first live birth 1982–2012. The relationship between having had any ovarian stimulation since 2005 and breast cancer incidence was studied in a cohort of women born 1960–1992. The main results from each cohort were:

Parous women

- Among the 1,340,211 parous women in the cohort, 38,047 (2.8%) had given birth following ART.

- Just over two thirds (69.7%) of women with ART births and 6.7% of women with non-ART births had an infertility-related diagnosis in the NPR.
- In multivariable adjusted analysis, the incidence of breast cancer was lower among women with infertility-related diagnoses and no ART birth (HR 0.83, 95% CI 0.77–0.89) and among women with ART birth (HR 0.84, 95% CI 0.74–0.95) compared to women with no infertility or ART.
- Compared to women with infertility-related diagnoses but no ART birth, women who gave birth following ART did not have a higher incidence of breast cancer (adjusted HR 1.01, 95% CI 0.88–1.17).
- Adjusting for BMI at the start of the first pregnancy did not affect the estimates in the subgroup of women with information on BMI.

Women born 1960–1992

- Of the 1,877,140 women born 1960–1992, 39,469 (2.1%) had gone through COS for ART and another 26,232 (1.4%) had received ovulation induction treatment between 2005 and 2012.
- Infertility-related diagnoses were recorded in the NPR for 75.3% of women with COS treatment, 53.9% of women with ovulation induction and 5.8% of women with no ovarian stimulation.
- The incidence of breast cancer was not higher among women who received COS (adjusted HR 0.86, 95% CI 0.69–1.07) or among women who received other hormonal fertility treatments (adjusted HR 0.79, 95% CI 0.60–1.05), compared to women with no infertility-related diagnosis or ovarian stimulation.
- Compared to women with infertility-related diagnoses and no ovarian stimulation, the breast cancer incidence was not higher among women treated with COS (adjusted HR 1.03, 95% CI 0.82–1.30) or ovulation induction (adjusted HR 0.95, 95% CI 0.71–1.28).
- The results did not seem to differ between parous and nulliparous women, and the likelihood ratio test for interaction was not significant (*p* adjusted = 0.1847).

5.3 STUDY III

Study III investigated the incidence of ovarian cancer and BOT among women with and without a diagnosis of infertility or ART treatment, in the cohort of parous women. The main results were:

- A diagnosis of infertility was recorded in the NPR for 66.3% of women with ART births and 3.8% of women with non-ART births.
- Women who had given birth following ART had a higher incidence of ovarian cancer, both compared to women with no infertility or ART (adjusted HR 2.43, 95% CI 1.73–

3.42) and compared to infertile women who conceived without ART (adjusted HR 1.79, 95% CI 1.18–2.71).

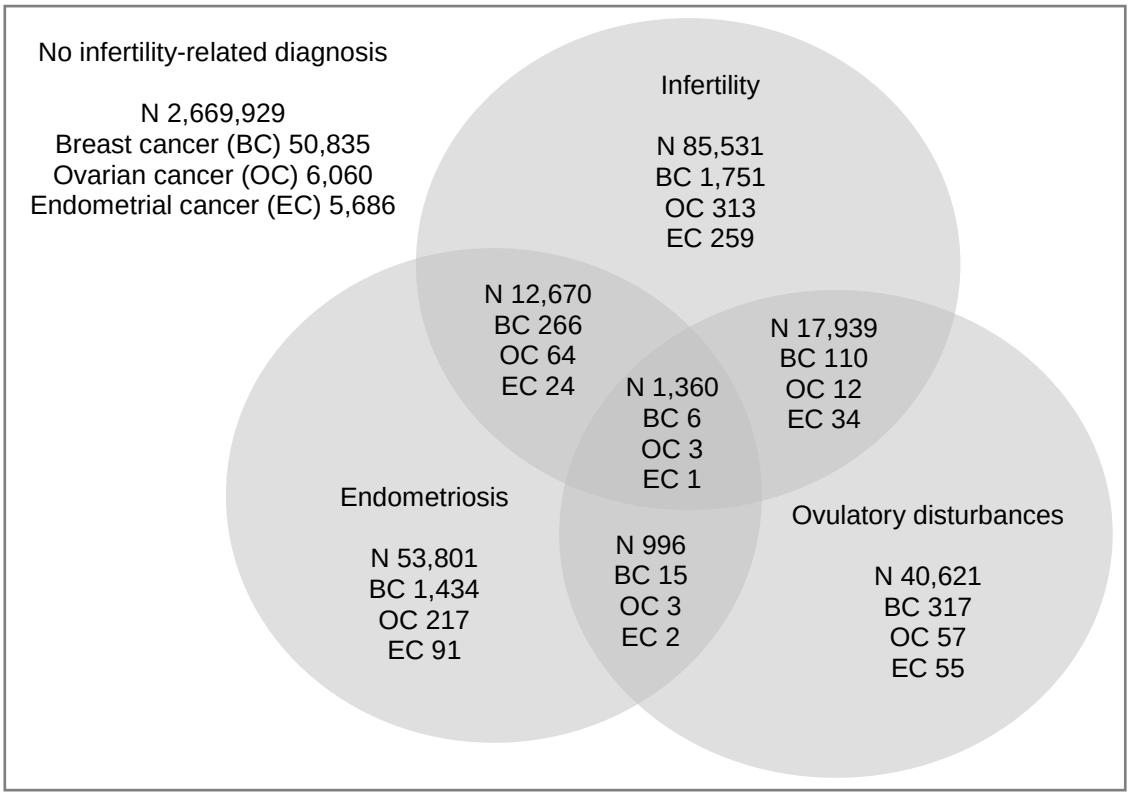
- The incidence of BOT was higher among women with ART birth when compared to women with no infertility or ART birth (adjusted HR 1.91, 95% CI 1.27–2.86). When comparing to infertile women with non-ART birth, the association was not statistically significant (adjusted HR 1.48, 95% CI 0.90–2.44).
- Excluding the first year of follow-up did not substantially change the results of the main analyses for either ovarian cancer or BOT.
- Neither BMI nor smoking before pregnancy appeared to influence the main results.

5.4 STUDY IV

Study IV investigated the relationship between infertility, with and without ovulatory disturbances or endometriosis, and incidence of cancer among women born 1942–1992. The main results were:

- Of the 2,882,847 women in the cohort, 4.1% had a diagnosis of infertility. Among infertile women, 16.4% had been diagnosed with ovulatory disturbances and 11.9% with endometriosis. Among non-infertile women, the corresponding figures were 1.5% and 2.0%, respectively. The overlap between these diagnoses and the number of incident cancer cases in each group are presented in Figure 5.2.

Figure 5.2 Venn diagram of exposure overlap and number of incident cancers in study IV.



- The incidence of ovarian cancer was higher among women with ovulatory disturbances (adjusted HR 1.53, 95% CI 1.18–1.98), endometriosis (adjusted HR 1.77, 95% CI 1.53–2.05) and infertility (adjusted HR 1.53, 95% CI 1.36–1.72), compared to women with none of these diagnoses. The highest incidence of ovarian cancer was seen in women diagnosed with both infertility and endometriosis (adjusted HR 2.19, 95% CI 1.70–2.82).
- In analyses stratified by either parity or age, the association between ovulatory disturbances and ovarian cancer was only significant among nulliparous women and before age 50, respectively. The associations with endometriosis and infertility remained significant also among parous women as well as in older ages.
- Women diagnosed with either infertility (adjusted HR 1.21, 95% CI 1.07–1.38) or ovulatory disturbances (adjusted HR 1.45, 95% CI 1.12–1.88) had a higher incidence of endometrial cancer, and the highest incidence was found in women with both these diagnoses (adjusted HR 2.90, 95% CI 2.05–4.08).
- The incidence of endometrial cancer was not higher among women exclusively diagnosed with endometriosis (adjusted HR 0.94, 95% CI 0.76–1.17), or in combination with infertility (adjusted HR 0.87, 95% CI 0.58–1.30).
- Being diagnosed with both infertility and ovulatory disturbances was associated with a higher incidence of endometrial cancer in both nulliparous (adjusted HR 3.46, 95% CI 2.22–5.38) and parous women (adjusted HR 2.28, 95% CI 1.32–3.93). A diagnosis of either ovulatory disturbances (adjusted HR 2.20, 95% CI 1.51–3.20) or infertility (adjusted HR 1.28, 95% CI 1.08–1.53) was associated with a higher incidence of endometrial cancer only among nulliparous women.
- When stratifying by age, the higher incidence of endometrial cancer among women diagnosed with infertility and/or ovulatory disturbances was only present below the age of 50. None of the infertility-related diagnoses were associated with a significantly higher incidence of post-menopausal endometrial cancer.

6 DISCUSSION

6.1 BREAST CANCER RISK

Neither infertility nor ovarian stimulation seem to increase the risk of breast cancer, although these factors may have an influence on mammographic density.

There was no evidence of an increased breast cancer risk among infertile women in studies II and IV. While these findings are in agreement with many previous studies (45,91,108–110), others have reported higher risks associated with infertility (38–40), endometriosis (41,42) and PCOS (43,44). However, none of the studies reporting a higher risk included adjustment for parity. Since nulliparity is a risk factor for breast cancer that is more common among infertile women, these studies may have overestimated cancer risk in this population.

Similar to the findings of most previous studies (38,45–50), women who had gone through ovarian stimulation did not have an increased risk of breast cancer in study II. In contrast to these results, a Norwegian study found a higher risk of breast cancer among women with ART births compared to other parous women (53). Since having an ART birth was not associated with a higher risk compared to either infertile or non-infertile parous women in study II, there may have been other differences between the respective study populations in Sweden and Norway.

Two recent meta-analyses have concluded that ART treatments do not seem to influence breast cancer risk (9,52), although one of them reported a slightly higher risk of breast cancer when restricting analyses to non-ART treatments of infertility performed before 1980 (9). However, this finding was based on only three studies and was not adjusted for parity. A Swedish study based on an historical cohort of infertile women reported a higher risk specifically among women who had gone through four or more cycles of high-dose clomiphene citrate stimulation (24). Analyses of breast cancer risk following ovulation induction in study II were based on treatments performed 2005 or later. These results may not be representative for women who received high doses of hormonal stimulation before 1980 since it is likely that treatment indications, drug types and dosage have changed between the two time periods.

Two previous studies have found a higher risk of breast cancer following gonadotropin use specifically among women who remain nulliparous after treatment (54,55). In study II, neither nulliparous nor parous women had an increased risk of breast cancer following ovarian stimulation, based on a mean follow-up of six to seven years. While it is possible that this follow-up was too short to identify an increase in long-term risk, studies on oral contraceptive and hormone replacement therapy use have only found increased risks of breast cancer linked to current or recent use (31).

The results from study I indicated that infertile women had slightly more dense tissue in the breast. Among infertile women, ART was associated with a higher dense volume compared to receiving no hormonal treatment. While this could implicate a long-term effect of ART

treatments on the breast tissue, it may also be explained by differences in the underlying infertility between women who had been treated with ART and those who had not. Additionally, the observed differences were small and it remains unclear whether they could influence breast cancer risk. A recent study on mammographic density reported that the risk of breast cancer increased by 3% for every 10 cm^3 higher absolute dense tissue, measured using the same method as in study I (61). Based on these results, the higher absolute dense volume in study I among women who had gone through ART could potentially correspond to a 1% increased risk of breast cancer.

6.2 OVARIAN CANCER RISK

Overall, infertile women had a higher risk of ovarian cancer than non-infertile women in studies III and IV. Ovarian cancer risk was higher among women diagnosed with infertility, endometriosis, and in a subpopulation of women with ovulatory disorders in study IV, compared to women with none of these diagnoses. In study III, women who had given birth following ART treatments had a higher risk of ovarian cancer and BOT.

Most previous studies have reported standardized incidence ratios (SIRs) of ovarian cancer among infertile women compared to the general population (38–40,45,83,111). Among these, all but two (38,45) found higher risks among infertile women. In addition, a cohort study in the United States reported a higher risk of ovarian cancer among infertile women (73). Only one of these studies adjusted for parity (39), while two case-control studies found no increased risks among infertile women when adjusting for parity (77,78). However, the risk of ovarian cancer was higher among infertile women even after adjusting for parity in study IV, implying that other aspects of infertility contributed to the increased risk in this population.

Women diagnosed with endometriosis had a higher risk of ovarian cancer in study IV. This is consistent with the findings of a meta-analysis from 2014 (112) as well as several recent studies (41,68,91,113). The mechanism behind this association is not entirely understood, though it is likely related to oxidative stress caused by retrograde menstruation as well as changes in the microenvironment that promotes carcinogenesis (114). The risk of ovarian cancer was also higher among nulliparous women diagnosed with ovulatory disturbances. So far, most previous studies have not found any increased risk in women with PCOS (44,110,115,116), although one study reported a higher risk of ovarian cancer among women with irregular menstruation (72).

In study III, the risk for ovarian cancer was increased in women with ART-births. Most previous studies have not found a higher risk of ovarian cancer following ART (46–48,50,68,75,76), including the few that compared the risk to that of infertile women (47,68,76). The results from study III also indicated that part of the association was due to the underlying infertility since the effect was smaller compared to other infertile women.

The higher risk following ART should also be considered in relation to the low incidence of ovarian cancer in the general population. In Sweden, five out of 1,000 women are expected to be diagnosed with ovarian cancer before 65 years of age (117). Based on this number, the higher incidence rates in study III would correspond to an absolute risk of seven in 1,000 among infertile women and eleven in 1,000 among women who have gone through ART treatment, before age 65. Accordingly, the identified difference in risk between infertile women conceiving with and without the help of ART is only four in a thousand.

The risk of BOT was also higher following ART births when compared to parous women with no infertility, similar to the results of a recent study from Norway (50). When comparing to infertile women with non-ART births the risk was not significantly higher after ART, indicating that the higher risk was confounded by the underlying cause of infertility. However, the point estimate was close to 1.5 suggesting that the study might have been underpowered to identify a modest increase in risk. Two previous studies have reported higher risks of BOT following ART treatments after adjusting for parity (76,87).

6.3 ENDOMETRIAL CANCER RISK

The results from study IV showed that endometrial cancer risk was higher among women diagnosed with infertility or ovulatory disturbances, but not in women with endometriosis, compared to women with none of these diagnoses.

The risk of endometrial cancer was higher in infertile women overall, with a stronger association among nulliparous women. These results are in line with those of a pooled case-control study that found infertility and nulliparity to be independently associated with endometrial cancer risk (89). Some previous studies have reported higher SIRs of endometrial cancer in infertile women when adjusting for age and calendar period (38,40,45), while one study found no increased risk after adjusting for parity (118).

Endometrial cancer risk was higher in women diagnosed either with infertility or ovulatory disturbances, and women with both of these diagnoses appeared to have the highest risk. This increased risk is most likely due to PCOS, which has consistently been found to be associated with endometrial cancer (44,110,116,119). Since PCOS is a common cause of infertility, this disorder likely contributed to the higher risk of endometrial cancer seen among women diagnosed with infertility alone.

There was no evidence of an increased risk of endometrial cancer among women with endometriosis. These findings are consistent with several previous studies (42,91,92,120). A few studies have reported that women with endometriosis have a higher risk of endometrial cancer (41,90,121,122). The studies have different methodological approaches and designs that could explain the contradictory results.

It was not possible to separate the potential effects of fertility treatments from that of infertility and related diagnoses in study IV. It is reasonable to assume that many women diagnosed with infertility also received some fertility treatment. A recent systematic review

reported that high doses of clomiphene citrate stimulation were associated with a higher endometrial cancer risk when comparing to other infertile women (93). Nevertheless, the authors noted that the association was likely confounded by the underlying cause of infertility since clomiphene citrate is commonly used to treat infertility due to PCOS. Further, a meta-analysis of six studies found no increased risk of endometrial cancer following fertility treatments (123).

6.4 METHODOLOGICAL CONSIDERATIONS

A key strength of the studies in this thesis is the use of national health and population registers with essentially complete coverage, enabling the identification of large study cohorts with reliable information on fertility treatments, cancer incidence and many important confounding factors. Another key strength is the utilization of survival analysis that accurately accounts for differences in age between exposure groups as well as loss to follow-up, while also allowing for correct adjustments of changes over calendar period.

6.4.1 Choice of comparison group

Many previous studies have compared the risk of cancer in infertile women to the expected number of cases in the general population by calculating SIRs. The expected number of cases is usually calculated on the basis of age and calendar period. Therefore the resulting estimates will not be adjusted for other risk factors, most importantly parity. Since several risk factors for cancer are more common among infertile women, comparisons to the general population may overestimate the effect of treatment itself.

In all of the included studies, the first comparison group was women without known fertility problems from the same cohort. While these models can be adjusted for parity and other confounders, the comparison cannot be used to separate potential effects of fertility treatments from those of infertility. In order to distinguish between these factors, a separate comparison group of infertile women with no known ovarian stimulation was identified in studies I-III. This comparison can give a better estimate of a potential treatment effect, assuming that causes of infertility are similarly distributed among women with and without treatment. However, the causes and severity of infertility may differ between the groups potentially causing residual confounding.

6.4.2 Information bias

Study I was mainly based on data from questionnaires that were filled out at the time of mammographic screening. This study design may cause recall bias, if the accuracy of the exposure information differs depending on the outcome. Since women were not aware of their mammographic density measures, it seems unlikely that it had any influence on the questionnaire answers. Most women will probably remember if they have ever gone through infertility and fertility treatments. However, older women may be less likely to recall the specific drug used in ovarian stimulations that were performed a long time ago, potentially causing some misclassification of treatment types.

In studies II-IV, information from the NPR was used to identify women with infertility and related diagnoses. As the NPR only covers in-patient care prior to 2001 some infertile women were likely misclassified as non-infertile, which could bias the estimates towards the null for infertility. Given the large population of non-infertile women in the comparison group, any such effect is likely to be small. However, some causes of infertility, such as ovulatory disturbances, are more likely to be diagnosed in out-patient care and may therefore have been more common among women with ART births than in the infertile comparison group.

In studies II and III, women who had ART treatments outside of Sweden or conceived spontaneously following an unsuccessful ART cycle were likely classified as having non-ART births. These misclassifications could bias the estimates towards the null, although likely to a small extent.

In study II, information on any dispensations of ovarian stimulation drugs was obtained from the PDR. Since the PDR was only available from July 2005, women treated before then and not after were classified as untreated. In addition, no information on whether the women actually took the medication was available and some untreated women may have been categorized as treated. Any such misclassification could bias the estimates towards the null.

Information bias can also be caused by differential misclassification of the outcome. Increased surveillance following ART could potentially influence incidence rates due to earlier detection. However, the mean age at diagnosis of BOT and ovarian cancer was not lower in exposed than unexposed women in study III. Further, since there is no screening program for ovarian cancer in Sweden, this is unlikely to explain the higher risk after ART.

6.4.3 Selection bias

Selection bias occurs when the distributions of exposure among cases and/or controls are different from that of the population.

In study I, non-responders could be a potential cause of selection bias. However, as the Karma cohort was established for the broader purpose of risk prediction of breast cancer and the questionnaire measures a wide range of different factors, it is unlikely that previous infertility or fertility treatments would influence women's decision to participate.

In studies based on population registers, selection bias can be caused by differential loss to follow-up. Although between 4 and 6% of the women in studies II-IV emigrated from Sweden during the respective study periods, it seems unlikely that emigration would be related to whether or not a woman will experience infertility or require fertility treatments.

6.4.4 Confounding and mediation

Factors that are associated with cancer and infertility or fertility treatment could bias the results of the included studies if they are not adjusted for. Each covariate included in the

analyses was chosen a priori based on the current knowledge of their potential influence on both the exposure and outcome.

Through linking of the national population-based registers, several important potential confounders were identified and included in the studies, including age, parity, education, calendar period and country of birth.

Age is the most important risk factor for the cancers in this thesis. In study I, age was included as a categorical variable to allow for non-linear associations across different age groups. In studies II-IV attained age was used as time-scale, which is the most efficient way to adjust for this confounder in a Cox model (124).

Calendar period was included to indirectly adjust for unmeasured factors that have changed over time, such as ovarian stimulation treatment protocols and cancer screening programs. Similarly, education level and country of birth are not confounders per se but markers of other factors that may influence the results such as social inequality and health seeking behavior.

Parity was included in all multivariable adjusted models as it is strongly linked to infertility, mammographic density and cancer risk. Since parity could also be an effect modifier, analyses that included nulliparous women were stratified by parity.

The questionnaire in study I included information on age at menarche, menopausal status, BMI, cigarette smoking and alcohol consumption at the time of mammography. Adjusting for BMI attenuated the association between infertility and absolute dense volume, while the other factors had very modest effects, if any.

BMI and smoking during pregnancy was recorded in the MBR for a subset of parous women in studies II and III. Models adjusted for BMI were included as sensitivity analyses in both studies, while models adjusted for smoking during pregnancy were added as sensitivity analyses in study III. Neither of these factors appeared to influence the associations between infertility, fertility treatments and cancer risk. However, high BMI could act as a mediator between ovulatory disturbances and endometrial cancer risk since PCOS may cause weight gain (19).

Age at first birth was not included in study I. The associations in parous women may have been mediated by age at first birth, as infertility problems are likely to delay childbearing and women who give birth later generally have higher mammographic density (62). When designing studies II-IV, age at first birth was included in order to assess associations that were not mediated by this factor.

In studies II-IV unmeasured factors that could cause residual confounding include ovulation induction treatments, age at menarche and menopause, oral contraceptive use and cause of infertility.

Since ovulation induction is the first-line treatment for anovulatory infertility, some of the women in study II and III had likely received clomiphene citrate and/or low-dose gonadotropin stimulation. This could potentially overestimate the effect of ART when comparing to non-infertile women. Assuming that ovulation induction was more common among infertile women with non-ART births, it could bias the results towards the null when comparing women with ART births to this group. However, most previous studies have not found any link between ovulation induction and the risk of breast or ovarian cancer (8).

Early age at menarche is a risk factor for breast and ovarian cancer, and a few studies have suggested that late menarche is associated with a slightly higher risk of infertility (125,126). Confounding by age at menarche could therefore have underestimated the associations in study IV.

Menopause occurs after the reproductive period and is not technically a confounder. Some studies have suggested that pre- and postmenopausal breast cancer may have different risk factors (127,128). To investigate whether the studied associations differed by menopausal status, age 50 was used as a proxy for menopause in studies II and IV.

Oral contraceptives could potentially have confounded the results of study III if infertile women used them less, as they are known to lower the risk of ovarian cancer. However, oral contraceptive use is unlikely to differ between groups before attempting to get pregnant, and they are also prescribed as treatment for PCOS and endometriosis.

6.4.5 Random error

In general, utilizing large cohorts should minimize the risk that the results of the included studies were influenced by random error. However, the small number of exposed cases led to imprecise estimates in analyses of BOT risk following ART treatments, as well as ovarian cancer risk among women diagnosed with ovulatory disturbances and infertility. Random error can result in either an over- or underestimation of the true effect.

6.4.6 Other limitations

For most of the study period (1982–2004), there was no information on ART treatments that did not lead to live births nor the stimulation protocol used. Therefore, it was not possible to study cancer risk in relation to number of ART cycles or total gonadotropin dose. The presence or absence of a dose-response relationship could have provided useful clues in determining whether the associations between ART treatments and ovarian tumor risks were due to the treatment or the underlying infertility.

6.5 GENERALIZABILITY

While ART treatments have been performed in Sweden for more than 30 years, few women received the treatment during the first decade, so the majority of women who have gone through ART are still young. In study II and III, there were no diagnoses of cancer above the

age of 60 among women with ART births. Therefore, the results from these studies may not be generalizable to women of older ages.

Although the total follow-up time was longer in study IV, infertility and related diagnoses were more common in recent years due to the improved coverage of the NPR. Since follow-up time was shorter in some exposure groups, especially for women diagnosed with ovulatory disturbances, the results for post-menopausal cancer risks were imprecise. The lack of significant results in women over 50 should not be interpreted as evidence that there is no association in this age group as it could also be due to a lack of power.

Only information on ART treatments that resulted in live births was available between 1982 and 2005. On average, 60-70% of couples who receive ART treatments succeed in having a child (129) and many women who go through these treatments will remain childless. Since having children lowers the risk of both breast and ovarian cancer, it is possible that an increased risk from treatment may be masked by the protective effect of parity. In study II, it was possible to investigate breast cancer risk following any ovarian stimulation by using information from the PDR, with a follow-up of eight years. This analysis could not be repeated for ovarian cancer or BOT due to the small number of exposed cases and the results from study III might not be representative for women who remain nulliparous after treatment.

7 CONCLUSIONS AND IMPLICATIONS

Neither infertility nor ovarian stimulations seem to increase the risk of breast cancer. Breast cancer risk was not higher among women diagnosed with infertility, endometriosis or ovulatory disturbances than in women with none of these diagnoses. Further, women treated with ART did not have a higher breast cancer risk compared to women who had not gone through treatment. These findings are especially reassuring since breast cancer is the most common cancer type in women.

Overall, infertile women have a higher risk of ovarian cancer than women with no infertility-related diagnoses. Endometriosis was consistently associated with a higher risk of ovarian cancer, both in parous and nulliparous women. In addition, ovulatory disorders may be associated with a higher risk of ovarian cancer specifically among nulliparous women, although the reasons for this association are unclear.

Women who have gone through ART have a higher risk of ovarian cancer and borderline ovarian tumors. Although the risk following ART was compared to that of other infertile women, it is still possible that this association was confounded by the underlying causes of infertility. Ovarian cancer is a rare disease and the absolute increases in risk were small. Since the risk of being diagnosed with ovarian cancer is still very low among women who have received ART, the findings should not be a cause for concern for individual infertile women or treating physicians. Nevertheless, the results highlight the importance to continue investigating cancer incidence in this population.

Endometrial cancer risk is higher in women with ovulatory disorders, especially in combination with infertility. This is likely due to PCOS, since this disorder is known to increase endometrial cancer risk through high levels of estrogen without counteraction by progesterone. Women with endometriosis did not have a higher risk of endometrial cancer.

In summary, breast cancer risk is not likely to be influenced by infertility or fertility treatments, while ovarian and endometrial cancer are more common in some groups of infertile women. Further, ART treatments may be associated with a small increase in the risk of ovarian cancer and borderline ovarian tumors.

8 FUTURE PERSPECTIVE

Despite their shortcomings, observational studies will continue to play an important role in this field of research. It is difficult, if not impossible, to perform clinical trials investigating long-term cancer risk.

ART treatments are performed during a woman's reproductive years while cancer is more likely to be diagnosed in older women. Even longer follow-up time will be required to fully investigate the possible long-term risks of ART.

Although the studies in this thesis are some of the largest to date, some analyses were still limited by low power. One way to overcome this problem is to perform analyses on pooled data. Nordic collaborations are promising since these countries have similar health care systems as well as population and health registers.

Many new opportunities in this research field are opening up thanks to clinical registers with improved coverage and more detailed information. With the introduction of specialized ART registries in Sweden and many other countries, it is now possible to include unsuccessful cycles as well as drug type and dosage. Future studies will therefore be able to investigate potential dose-response relationships between different stimulation protocols and cancer risk in both parous and nulliparous women.

In order to further disentangle potential effects of treatment, future studies should also include comparison groups of infertile women. In Sweden, the introduction of ICD-10 and the inclusion of specialist out-patient care in the NPR has resulted in better coverage and more detailed information on infertility than previously. Using this information, future studies may be able to adjust for specific types of infertility or to stratify analyses by infertility cause in order to identify whether fertility treatments may influence cancer risk. However, infertility is generally not investigated further than what is needed to decide on a treatment plan and infertility is commonly diagnosed as unexplained also in recent years.

Since different cancer types may have different risk factors, it would be interesting to investigate risks separately for cancers of different morphology. Using information from the national quality registries for specific cancer types can provide further details about the cancer diagnoses. Cancer stage at diagnosis among exposed and unexposed women could also be compared in order to more thoroughly investigate potential surveillance bias following fertility treatments.

Studies on prospective cohorts, such as the Karma study and the Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study (130) in Sweden, may give more insight into the influence of specific confounders not included in the national registers, since they can include more in-depth information on factors such as life style.

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